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Trypanosomes from North American Amphibians, with a Description of *Trypanosoma grylli* Nigrelli (1944) from *Acris gryllus* (Le Conte).

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(Plate I; Text-figures 1-4).

INTRODUCTION.

The North American continent is the natural habitat of many species of amphibians, yet relatively few have been examined for their blood parasites. Table I lists the species that have been examined for trypanosomes.

The extreme polymorphism demonstrated by many of these trypanosomes makes them very difficult to classify on a morphological basis alone. Until cross-infection experiments are made to prove them otherwise, the species described by various investigators must remain valid.

The present contribution deals with trypanosomes found in certain urodeles and anurans from different parts of the eastern and southern United States, together with a more complete description of *Trypanosoma grylli* Nigrelli (1944) from cricket frogs caught in Georgia.

MATERIAL AND METHODS.

Table I also includes those species examined by the writer. These amphibians were collected from Connecticut, New York, New Jersey, Pennsylvania, North Carolina, Georgia, Florida, Michigan and Mississippi.

Blood smears were taken as the amphibians were received in the laboratory and stained immediately with Wright's and Giemsa's blood stains. Splenic smears also were made to determine whether or not intracellular leishmanian bodies were present.

Efforts at cultivating trypanosomes (*T. diemyctyli*) from *Triturus viridescens* were unsuccessful, although it was found possible to isolate the trypanosomes under sterile conditions. Further experiments are under way along this line and attempts will also be made to inoculate laboratory-raised amphibians with *Triturus*-infected blood to establish the specificity of this trypanosome.

Trypanosoma grylli Nigrelli, 1944.

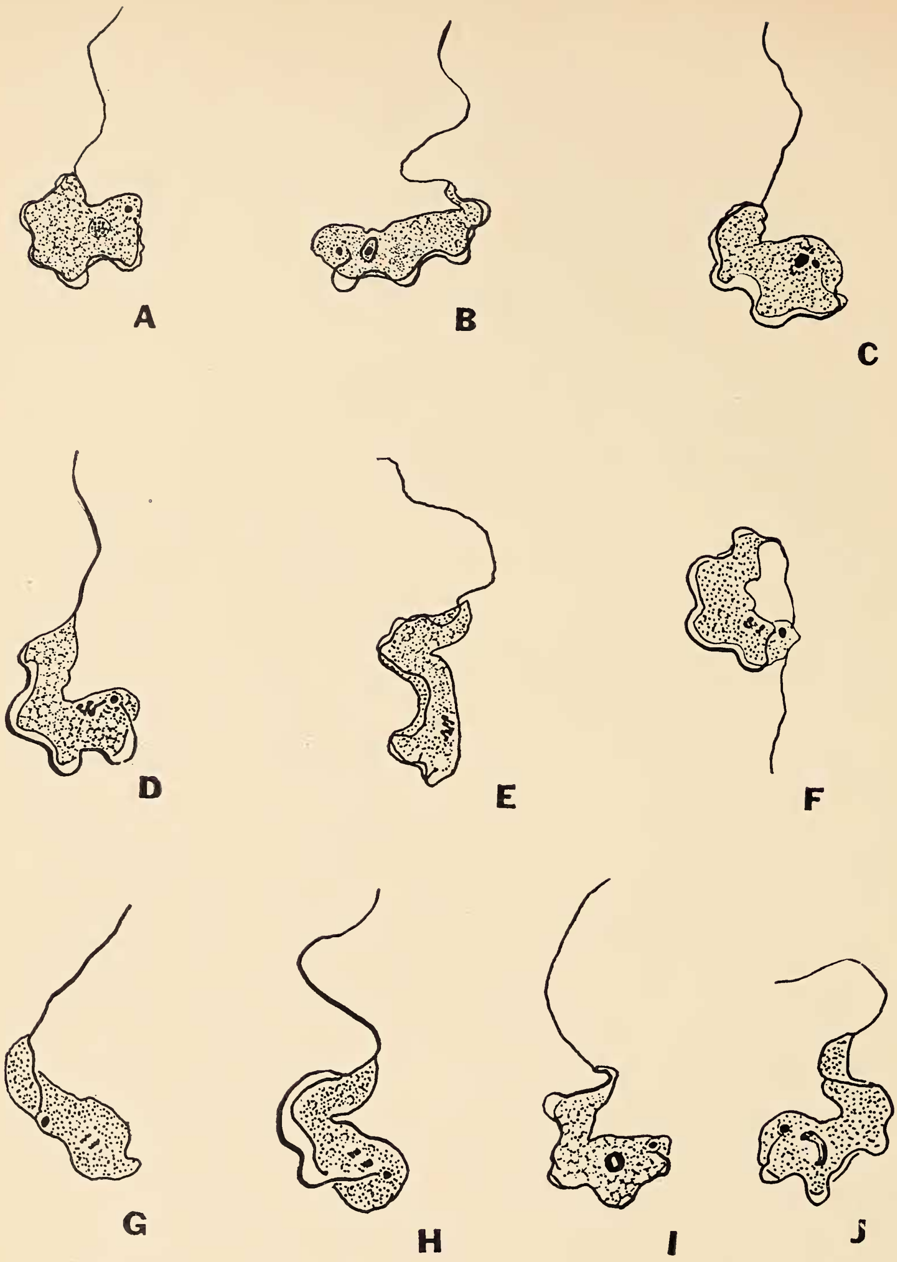
(Text-figure 1 A-J).

A short preliminary description of this flagellate was given by the writer in 1944. Twenty-four frogs collected from Georgia were found to be 100% infected and in all cases the infection was relatively heavy. Unlike most of the trypanosomes of amphibians, *T. grylli* is a monomorphic species in which the posterior end is round, while the rest of the body is variable in shape. Fifty trypanosomes were measured, showing a variation from 15.77 to 19.92 μ in length (average 17.43) and from 4.15 to 6.64 μ in width (average 5.31). The parabasal body is well developed, surrounded by a clear area. It is posterior in position, with the nucleus lying nearby. The blepharoplast, from which the intracellular axoneme arises, is present. This fibril passes anteriorly as the border of the undulating membrane and terminates in a well developed flagellum. The length of the flagellum may be as long or slightly longer than the length of the body of the parasite. The periplast is weakly developed, which may account for the amoeboid-like movements and the shape taken by this trypanosome. The cytoplasm is highly vacuolated and no internal myonemes were seen. In the vegetative nucleus a well developed karyosome with a peripheral ring of chromatin material is present. As may be noted from an examination of the figures of *T. grylli*, many of the nuclei are in various phases of mitosis.

TRYPANOSOMES FROM HYLIDAE.

(Text-figure 2 B-F).

Both the incidence and intensity of trypanosome infection in various species of Hylidae examined were very low. Three flagellates were found in smears from two *Hyla andersoni* (Figs. D-F). The presence of a crithidia-like form (Fig. E) and differences in the position of the parabasal body indicates that the infection is a recent one. The parasites measure 10-18 μ in length and 2-3 μ in width. The undulating membrane is fairly well developed with the terminating flagellum being shorter than the length of the body.

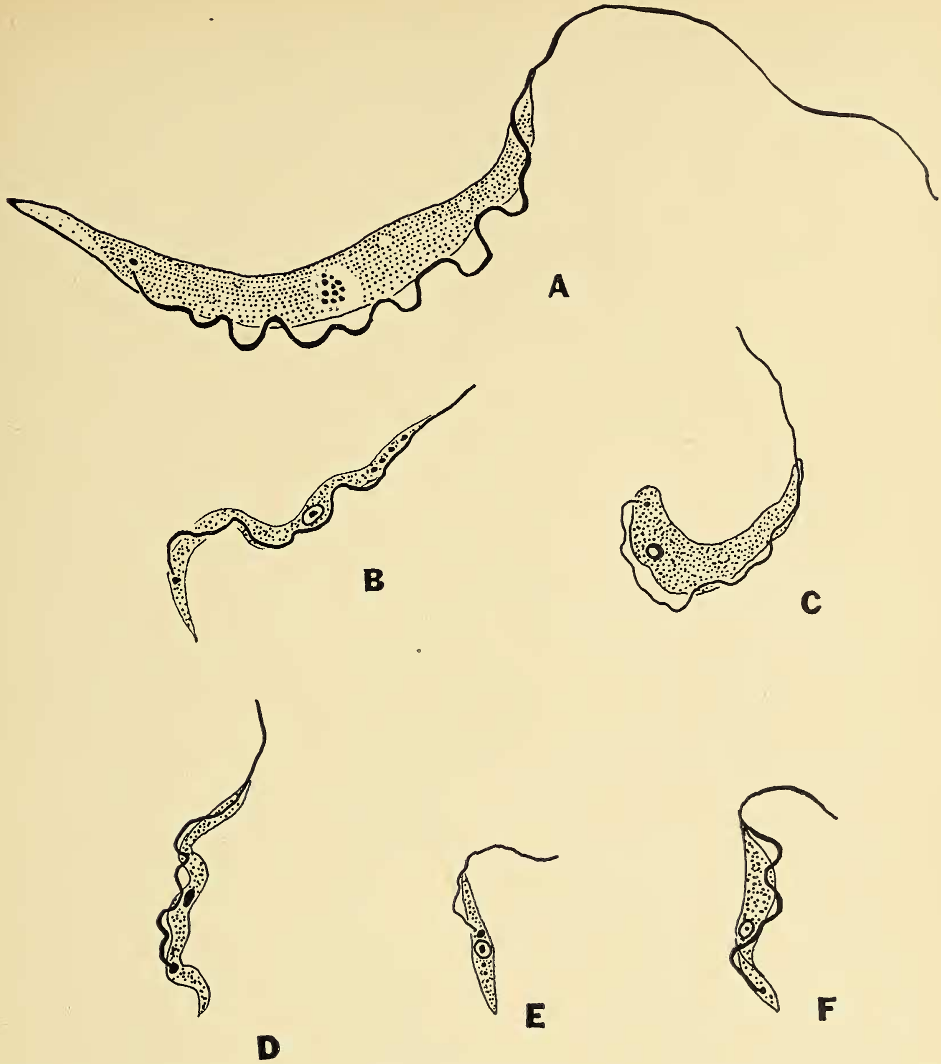


TEXT-FIG. 1. A-J. *Trypanosoma grylli* Nigrelli (1944) from *Acris gryllus.* Note the relationship of the nucleus with parabasal body, the highly metabolic body and nuclei in various stages of mitosis. × 1200.

The trypanosome (Fig. B) found in *Hyla versicolor* is long and slender in form, with the parabasal body a short distance from the posterior end. The nucleus is centrally located and the cytoplasm granular in appearance. The undulating membrane is weakly developed and the free flagellum comparatively short. The parasite measures about 35 μ in length and about 2.3 μ in width.

The flagellate (Fig. C) from *Hyla crucifer* shows certain similarities to *T. grylli,* especially in appearance and in the location of the parabasal body. However, the length and width of this parasite is greater than any of the trypanosomes found in the cricket frog, measuring 27.2 by 10.8 μ.

The trypanosomes from these tree frogs are not named because of insufficient material for comparative study. They resem-



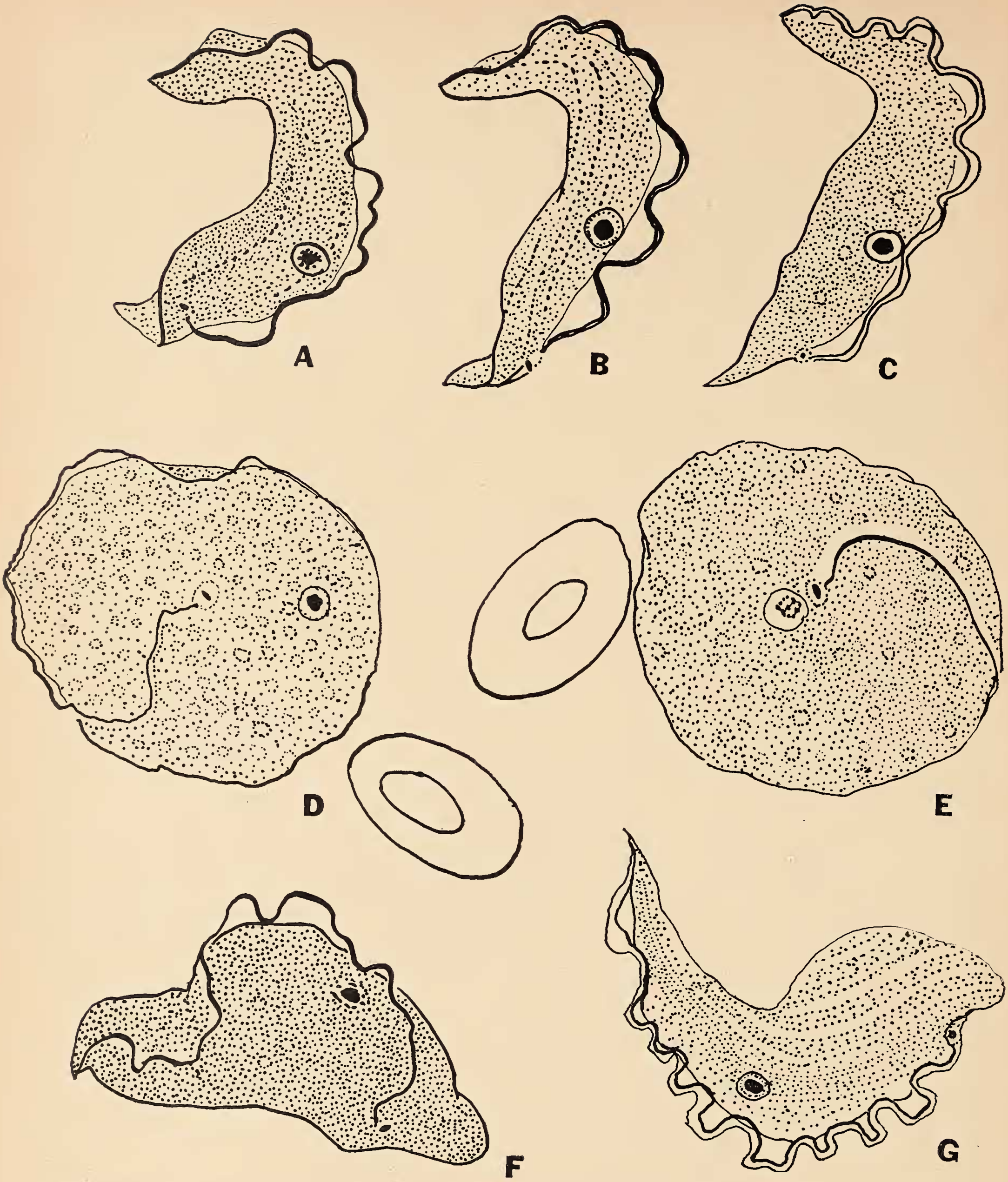
TEXT-FIG. 2. **A**. *Trypanosoma* sp. from *Rana pipiens*. **B** & **C**. *Trypanosoma* spp. from *Hyla versicolor* and *H. crucifer*, respectively. **D-F**. *Trypanosoma* sp. from *Hyla andersoni*. Note position of parabasal body and development of undulating membrane. Fig. **E** shows a crithidia form, indicating that the infection in this host is a recent one. × 1200.

ble somewhat the small elongate forms of *T. rotatorium* that have been reported from tadpoles from various parts of the world. Insofar as is known, Brandt (1936) was the first to report a species of trypanosomes from a species of North American Hylidae. He referred to the flagellates found in *Hyla crucifer* from North Carolina as *Trypanosoma rotatorium*.

TRYPANOSOME FROM *Rana pipiens*.

(Text-figure 2 A).

Trypanosomes recovered from smears taken from eight pickerel frogs measure 68-72 by 6-9 μ and resemble to a certain extent the long slender form of *T. diemyctyli* found in the newt (compare with Plate I; see also Nigrelli, 1929). The position of the nucleus and parabasal, the clear zone at the posterior tip of the body, the degree of development of the undulating membrane and the flagellum, and size of the body are characteristics which resemble those present in *T. diemyctyli*. Lack of sufficient material for a more detailed study makes it difficult to name this form. However, it is different from *T. rotatorium* and *T. inopinatum* Ed. & Et. Sergent (1904) reported from *Rana pipiens* by several investigators (see Kudo, 1922; Fantham, Porter & Richardson, 1942).

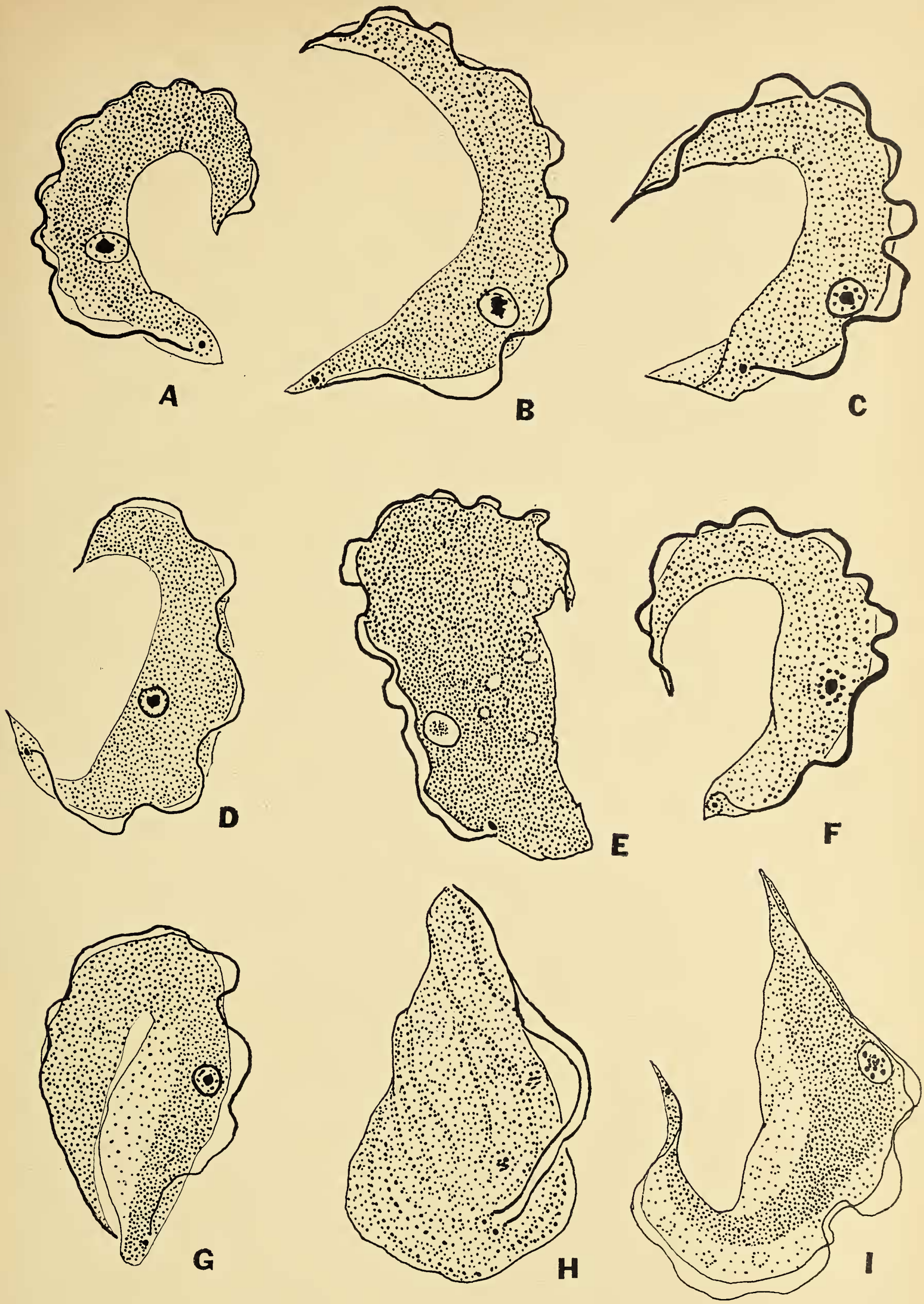


TEXT-FIG. 3. *Trypanosoma rotatorium* (Mayer) from *Rana clamitans*. Note the highly polymorphic nature of this trypanosome. Figs. **A-C**; **F & G**. Typical forms. Figs. **D** and **E** rounded dividing forms. Note the juxtaposition of nucleus and parabasal in the flagellate in Fig. **E**. × 1200.

Trypanosoma rotatorium (Mayer).

(Text-figures 3, 4).

Typical polymorphic forms of *Trypanosoma rotatorium* were found in *Rana clamitans* and *Rana catesbeiana* from various localities in Connecticut, New York, New Jersey and Pennsylvania. This flagellate has been reported from all the localities in which these hosts occur. In the present case, the trypanosomes from adult *R. clamitans* (Text-figure 3 A-G) are of two general types designated here as elongated forms and round forms, the former measuring 51-80 × 10-25 μ and the latter 48-50 × 45 μ. The trypanosomes from *R. catesbeiana* (Text-figure 4 A-I) were also variable in form but no round individuals were encountered.



TEXT-FIG. 4. *Trypanosoma rotatorium* (Mayer) from *Rana catesbeiana*. Figs. E, H, I show dividing individuals. × 1200.

They measure 51-85 × 12-19 μ. No free flagella were noted and stages in binary fission were seen frequently.

Several *Rana catesbeiana* were caught that had leeches (*Macrobdella*?) on them. Stained smears of the partly digested blood

TABLE I. North American Amphibians Examined for Trypanosomes.

AMPHIBIA	TRYPANOSOME	AUTHOR	YEAR
Acris gryllus (Le Conte)	*T. grylli*	Nigrelli	1944
Ambystoma maculatus (Shaw)	Negative	Nigrelli	Present paper
Ambystoma opacum (Gravenhorst)	Negative	Nigrelli	Present paper
Ambystoma tigrinum (Green)	Negative	Nigrelli	Present paper
Ambystoma tigrinum (Green)	Negative	Roudabush & Coatney	1937
Bufo americanus Holbrook	*T. lavalia*	Fantham, Porter & Richardson	1942
Bufo americanus Holbrook	*T. gaumontis*	Fantham, Porter & Richardson	1942
Bufo americanus Holbrook	*T. montrealis*	Fantham, Porter & Richardson	1942
Bufo fowleri (Hinckley)	*T. rotatorium*	Brandt	1936
Bufo woodhousii Girard	Negative	Roudabush & Coatney	1937
Cryptobranchus alleganiensis (Daudin)	*T. cryptobranchi*	Roudabush & Coatney	1937
Desmognathus fuscus (Rafinesque)	Negative	Nigrelli	Present paper
Desmognathus fuscus (Rafinesque)	Negative	Hegner	1921
Hyla andersoni Baird	*Trypanosoma* sp.	Nigrelli	Present paper
Hyla cinera (Schneider)	Negative	Nigrelli	Present paper
Hyla crucifer Wied	*T. rotatorium*	Brandt	1936
Hyla crucifer Wied	*Trypanosoma* sp.	Nigrelli	Present paper
Hyla femoralis Latreille	Negative	Nigrelli	Present paper
Hyla squirella Latreille	Negative	Nigrelli	Present paper
Hyla versicolor (Le Conte)	*Trypanosoma* sp.	Nigrelli	Present paper
Necturus maculosus (Rafinesque)	*Trypanosoma* sp.	Hegner	1921
Necturus maculosus (Rafinesque)	*Trypanosoma* sp.	Roudabush & Coatney	1937
Necturus maculosus (Rafinesque)	*Trypanosoma* sp.	Nigrelli	Present paper
Plethodon cinereus (Green)	*Trypanosoma* sp.	Hegner	1921
Plethodon glutinosus (Green)	*Trypanosoma* sp.	Hegner	1921
Pseudacris brimleyi (Brandt & Walker)	*T. rotatorium*	Brandt	1936
Pseudotriton rubra (Latreille)	Negative	Nigrelli	Present paper
Rana catesbeiana Shaw	*T. rotatorium*	Brandt	1936
Rana catesbeiana Shaw	*T. rotatorium*	Fantham, et al	1942
Rana catesbeiana Shaw	*T. rotatorium*	Nigrelli	Present paper
Rana catesbeiana Shaw	*T. inopinatum*	Fantham, et al	1942
Rana catesbeiana Shaw	*Trypanosoma* sp.	Hegner	1920[1]
Rana clamitans Latreille	*T. clamatae*	Stebbins	1907
Rana clamitans Latreille	*T. parvum*	Kudo	1922
Rana clamitans Latreille	*T. rotatorium*	Kudo	1922
Rana clamitans Latreille	*T. rotatorium*	Fantham, et al	1942
Rana clamitans Latreille	*T. rotatorium*	Nigrelli	Present paper
Rana clamitans Latreille	*Trypanosoma* sp.[2]	Stebbins	1907
Rana clamitans Latreille	*Trypanosoma* sp.	Hegner	1920
Rana palustris Le Conte	Negative	Nigrelli	Present paper
Rana pipiens Schreber	*T. rotatorium*	Kudo	1922
Rana pipiens Schreber	*T. rotatorium*	Packchanian	1934
Rana pipiens Schreber	*T. rotatorium*	Fantham, et al	1942
Rana pipiens Schreber	*T. inopinatum*	Fantham, et al	1942
Rana pipiens Schreber	*Trypanosoma* sp.	Nigrelli	Present paper
Rana sphenocephala (Cope)	*T. rotatorium*	Brandt	1936
Rana sylvatica Le Conte	Negative	Fantham, et al	1942
Rana sylvatica Le Conte	Negative	Nigrelli	Present paper
Rana virgatipes Cope	Negative	Nigrelli	Present paper
Scaphiopus holbrooki (Harlan)	Negative	Brandt	1936
Spelerpes (Eurycea) bislineatus (Green)	Negative	Hegner	1921
Triturus viridescens (Rafinesque)	*T. diemyctyli*	Tobey	1906
Triturus viridescens (Rafinesque)	*T. diemyctyli*	Hegner	1921
Triturus viridescens (Rafinesque)	*T. diemyctyli*	Nigrelli	1929
Triturus viridescens (Rafinesque)	*T. diemyctyli*	Nigrelli	Present paper

[1] See Calkins (1933).
[2] Larger of two forms found, probably *T. rotatorium*.

taken from the gut of the leeches showed the presence of many leptomonad and crithidia bodies in various stages of longitudinal fission, much like those reported by the writer (1929) for the metacyclic forms of *T. diemyctyli*. The cytoplasm of the parasites from the leeches contained red staining granules (volutin?) not unlike those that

TABLE II. Comparative Measurements of Trypanosomes from North American Amphibians

TRYPANOSOME	AVERAGE (microns)	WIDTH	LENGTH	FLAGELLUM
1. *T. rotatorium* (Mayer, 1843)				
Fantham, et al (1942)	47.4-72.6	3.0-26.7	......	Very short
Nigrelli (present paper)				
From adult *R. clamitans*				
Elongate forms	51-80	10-25	67.7×17.2	None or very short
Round forms	48-50	43-45	44×49	None or very short
From adult *R. catesbeiana*	51-85	12-19	67.7×12.8	None or very short
2. *T. inopinatum* Sergent & Sergent (1904)				
Original Measurements	16.5-21	1.5-2.2	......	1-6
Kudo (1939)				
Slender forms	12-20	......	......	......
Larger Forms	30-35	......	......	......
Fantham, et al (1942)	......	......	......	1.5-2.5
3. *T. diemyctyli* Tobey (1906)				
Hegner (1921)	38.1-75.3	1.9-4.4	56×2.89	......
Nigrelli (1929)				
Broad forms	63.5-79.4	5.2-9.0	71×8.2	Very long
Slender forms	38-57	1.9-4.5	53×3.8	
4. *T. clamatae* Stebbins (1907)				
Slender forms	21	2.5-2.8	......	12-13
Larger forms	27.56-47	16.78-28.51	......	5.96-14.79
5. *T. parvum* Kudo (1922)	11-14	1.2-1.9	......	5-15
6. *T. cryptobranchi* Roudabush & Coatney (1936)	46.8-77.4	1.8-5.84	60.87×3.51	31.61
7. *T. lavalia* Fantham, Porter, & Richardson (1942)	31.1-35.5	3.9-4.4	......	1.5-2.6
8. *T. gaumontis* Fantham, Porter & Richardson (1942)				
Group I	15-15.8	1.3-1.85	......	None
Group II	19.7-20.7	1.5-1.85	......	None
9. *T. montrealis* Fantham, Porter & Richardson (1942)	45-68	1.8-6	44-45	3-5.5
10. *T. grylli* Nigrelli (1944)	15.77-19.92	4.5-6.6	17.4×5.4	ca. 17

are known to occur in similar stages of other species. The presence of metacyclic forms in the gut of leeches is good evidence that these annelids are the true intermediate hosts of *T. rotatorium*, and presumably of the trypanosomes of some of the other Amphibia.

Trypanosoma diemyctyli Tobey from *Triturus viridescens*. (Plate I; Figures 1-4).

Details concerning the morphology and life-history of *T. diemctyli* Tobey (1909) were reported by the writer in 1929. This is a dimorphic species, involving long and broad forms, the former measuring 46-65 × 2.5-5.0 μ and the latter varying from 63-5-79 × 8.2 μ. The life cycle of *T. diemyctyli* was demonstrated experimentally. A leech is the transmitting agent. In the newt, reproduction takes place by binary fission while free in the blood stream, and in large mononuclear leucocytes where leishmanian bodies are produced. A similar cycle was demonstrated by Carini (1912) for *T. leptodactyli*, a form occurring in the South American amphibian, *Leptodactylus ocellatus*. Reproduction by formation of leishmanian bodies that are intracellular parasites is characteristic of *Trypanosoma cruzi*, the causative agent of South American human trypanosomiasis (Chaga's disease). However, in Chaga's disease, the trypanosomes do not multiply in the blood stream. Reproduction is limited to the intracellular stage.

DISCUSSION.

Table II lists and compares the various species of trypanosomes reported from North American amphibians. Whether or not they can all be relegated to one or more species cannot be answered at this time. It is certain that size and form alone are not good diagnostic characters to establish a species. The size of the host may have some influence on these factors.

Culturing the trypanosomes may throw some light on specificity. Characteristics such as shape and size of the colonies, the time it takes the colonies to develop, cyclic forms that may occur and nutritional requirements, would in all probability indicate species differences. Amphibian trypanosomes have been cultivated by several investigators, and Nöller (1913) obtained a good growth in sealed preparations of infected frog's blood mixed with an equal quantity of bouillon. He was able to follow the development of the large trypanosomes typical of *T. rotatorium* from *Rana esculenta* into the smaller, slender flagellates (crithidia) which result from repeated binary fission. Ponselle (1923) showed that the development of these large trypanosomes is dependent upon the reaction of the medium. He found that a mixture of broth (pH 6.3) and one-tenth its volume of defibrinated rabbit's blood yielded good cultures of *T. rotatorium* but would not support the growth of *T. inopinatum*, a second species found in *Rana esculenta*. However, the latter flagellate was grown in a mixture of equal parts of distilled water and defibrinated rabbit's blood, a mixture which, in turn, would not support the growth of *T. rotatorium*. Galliard (1926) was able to keep *T. inopinatum* alive for two years in sealed tubes of this medium. Packchanian (1934) cultured *T. rotatorium* from *Rana pipiens* on N.N.N. medium. He reported that the organisms formed colonies only after several months of cultivation, but once they began, they colonized readily thereafter. The colonies reached a size of about 8 mm. Varga & Bacsich (1938) obtained weak cultures on Zeisler's substrate but were able to find many dividing forms of *T. rotatorium*.

Cross-infection experiments and serological tests may also give some evidence as to the validity of the described species of trypanosomes. Nöller (1913) showed that infections in adult frogs (*R. esculenta*) may be superimposed by a second one by inoculation of infected blood from tadpoles of this host species, or by injection of large doses of cultured trypanosomes. Further, infection of *Rana temporaria* with cultured *T. rotatorium* was also accomplished. Under similar conditions the tree frog, *Hyla arborea*, was successfully infected, suggesting that *T. hylae* of França (1908) naturally found in this amphibian may be identical with *T. rotatorium*. Transmission experiments with toads (*Bombinator igneus*) were negative. This would indicate a natural immunity and a certain degree of host specificity.

The presence of more than one species of the same type of parasite in the same host is not uncommon. Thus, at least three species were found to occur in the *R. esculenta* (European green frog): *T. inopinatum* Sergent & Sergent (1904), *T. neveu-lemairei* Brumpt (1923) and *T. rotatorium* (Mayer). As is shown above, *T. inopinatum* and *T. rotatorium* appear to be distinct species since their culture requirements are different. In this country *T. clamatae* Stebbins (1907), *T. parvum* Kudo (1922) and *T. rotatorium* of various investigators, were reported from *Rana clamitans*. *T. parvum* and *T. clamatae* are probably the same and these, in turn, may be young stages of *T. rotatorium*. More recently, Fantham, Porter & Richardson (1942) found *T. inopinatum* in addition to *T. rotatorium* in *R. catesbeiana*. Here again, *T. inopinatum* may be a cyclic form of *T. rotatorium*. Only by cultivation on the differential test media of Ponselle can this assumption be proved or disproved. For *Bufo americanus* they describe three new species: *T. lavalia*, *T. gaumontis* and *T. montrealis*. The three occur in toads from different regions of Canada. However, they do not state whether or not a mixed infection of two or more species was encountered. The distinction between these species is vague, being separated mainly on differences in size.

It is perhaps through serological reactions that specificity of these amphibian trypanosomes may be determined. That this is a possibility has been demonstrated by several investigators for trypanosomes of warm blooded animals. It is known that some animals have sera with trypanolytic properties. Thus, human serum destroys all the pathogenic trypanosomes of mammals with the exception of those found in man (see Culbertson, 1941). The same results have been determined for other animals, and may also be true for the Amphibia. It is an established fact that anatomically related Amphibia show, in some instances, great divergence when tested serologically. Boyden & Noble (1933) have demonstrated by serological reactions that "Within the Salinetia, *Rana catesbeiana* and *Rana clamitans* are very close together, while *Rana pipiens* is not very close to either. *Hyla septentrionalis* is remote from all the species of Rana." On the same basis, relationships between certain of the Caudata were determined. *Triturus* and *Cryptobranchus* are not related. No mention was made of the serological affinities of the Bufonidae. There seems to be some correlation between these serological reactions and the species of trypanosomes harbored by the various amphibian hosts. It has always been felt among certain investigators, as indicated above, that the trypanosomes occurring in *R. catesbeiana* and *R. clamitans* are *T. rotatorium.* There is some doubt about the validity of *T. rotatorium* and *T. inopinatum* reported by several investigators (see Table I) from *Rana pipiens.* The flagellate reported by the writer from this species seems to be different from the highly polymorphic *T. rotatorium.*

Trypanosoma diemyctyli, T. cryptobranchi, T. grylli and the species described by Fantham, et al., from *Bufo americanus*, appear to be good species, and the correlation between the serological reactions of some of the hosts involved and their haemoflagellates is very striking. *T. diemyctyli* has always been considered a distinct species. *T. cryptobranchi* is a comparatively new species, but there seems to be no doubt about its validity. *T. grylli* is unlike any of the previously described trypanosomes from amphibians. Those found in *Bufo americanus* are probably valid, but it is the writer's opinion that the three species described from this host could be reduced to a single type. The trypanosomes recovered from various species of *Hyla* have not been designated because of insufficient material. Brandt (1936) categorically considered all the trypanosomes he found in the amphibians he investigated as *T. rotatorium.* Those present in *R. catesbeiana* and *R. sphenocephala* probably are but those in *Bufo fowleri, Pseudacris brimleyi* and *Hyla crucifer* may not be. All this material should be re-investigated to determine species differences, if any.

In any event the authenticity of the various species of trypanosomes in North American amphibians can only be established, in view of their highly polymorphic nature, by serological tests and by cultivation of the organisms found in the different host species. A simple experiment, which would add much to our knowledge of these trypanosomes, would be to test for the trypanolytic action of sera from various amphibians on cultured strains of a known species.

Summary.

1. Nineteen species of Caudata and Salinetia were examined for trypanosomes.

2. Infections were found in the following species: *Acris gryllus, Hyla andersoni, Hyla crucifer, Hyla versicolor, Rana catesbeiana, Rana clamitans, Rana pipiens* and *Triturus viridescens.*

3. The species found in *Acris gryllus* is considered new and designated as *Trypanosoma grylli* Nigrelli, 1944. A detailed description is given in this paper.

4. The species found in *R. catesbeiana* and *R. clamitans* is considered as *T. rotatorium* (Mayer); that found in *Triturus viridescens* as *T. diemyctyli* Tobey. The trypanosomes found in various species of *Hyla* and in *R. pipiens* were not named because of insufficient material to make a proper diagnosis.

5. A list of North American amphibians examined for trypanosomes by various investigators is given.

6. A table of measurements of the several species of trypanosomes reported is included in this study.

7. The apparent correlation between the serological reaction of the various hosts and the species of trypanosome harbored is discussed.

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EXPLANATION OF THE PLATE.

PLATE I.

Figs. 1-5. *Trypanosoma diemyctyli* from blood of *Triturus viridescens.* × 3200.

FIG. 1.

FIG. 2.

FIG. 3.

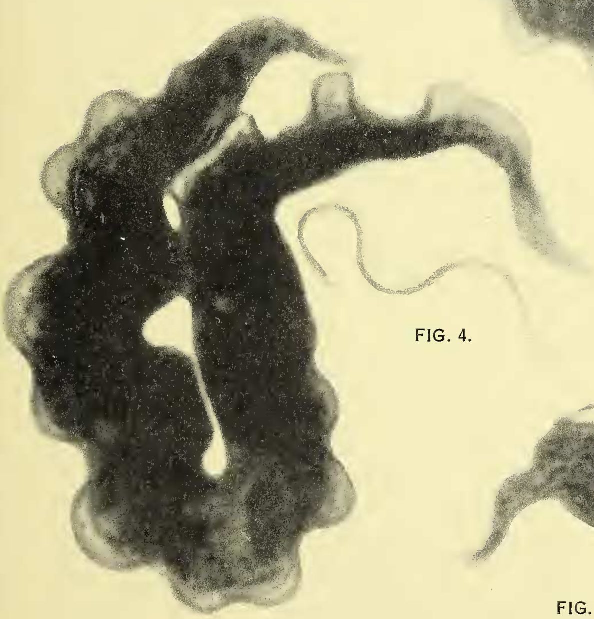

FIG. 4.

FIG. 5.

TRYPANOSOMES FROM NORTH AMERICAN AMPHIBIANS, WITH A DESCRIPTION OF TRYPANOSOMA GRYLLI NIGRELLI (1944) FROM ACRIS GRYLLUS (LE CONTE).